

Genetic Screening of Newborns in Baku for Hereditary Hemoglobinopathies

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Abstract

Hereditary hemoglobinopathies are included in a group of blood diseases called hemolytic anemias and this is a group of diseases of the hemoglobin of human erythrocytes. In the general case of hemoglobinopathies - the synthesis of abnormal hemoglobin. Hemolytic anemias occur in about 12% of the world's population. This article is devoted to the results of genetic screening among newborns in Baku in order to study the frequency of hemoglobinopathies. The study involved 296 newborns. Among them, 159 boys and 137 girls. The studies were carried out at the Central Clinical Hospital Neftchilarov mountains. Baku in 2018-2021, the results were processed in the genetic laboratory at the hospital. To determine hemoglobinopathies among newborns, we used the analytical method of iso-electrofocusing on polyacrylamide-ampholine plates with pH 3.5-9.5 and 5.5-8.5. This method allowed us to divide the hemolysate prepared from the blood of newborns into nine hemoglobin fractions: HbA1, HbA1c, HbA2, HbA3, HbF1, HbF2, methemoglobin, intermediate methemoglobin (in the form of two fractions). All results of the study are shown in 2 tables. Table 1 shows the results of genetic screening of blood of newborns for α - and β -thalassemia and levels of hemoglobin and methemoglobin fractions, table 2 shows the results of genetic screening of newborns for abnormal hemoglobins and shows the results screening levels of methemoglobin and intermediate methemoglobin in the blood of newborns. The article also details the results of molecular diagnostics of the β -thalassemia gene in newborns. To identify the type of mutations in β -thalassemia, genomic DNA was isolated from the venous blood of 4 newborns diagnosed with heterozygous β -thalassemia and their parents. The type of mutation in β -thalassemia was determined by high-temperature allele-specific amplification. For this, synthetic oligonucleotide probes of various contents were used. Figures 1 and 2 show electro-phoregrams of PCR diagnostics with a mutation and the replacement of the guanine nucleotide with the adenine nucleotide in various positions.

Keywords: screening, newborn, hemoglobin, globin, anemia, thalassemia.

Introduction

The second half of the 20th century is significant for biology and medicine with achievements in the field of molecular genetics, penetration into the deepest secrets of protein biosynthesis and molecular pathology. Hemoglobin and its abnormalities are recognized as the best model for research in this area [1].

Hereditary hemoglobinopathies are included in a group of blood diseases called hemolytic anemias and this is a group of diseases of the hemoglobin of human erythrocytes. Hemolytic anemias occur in about 12% of the world's population. In the general case, the synthesis of abnormal hemoglobin is called hemoglobinopathy. Hereditary hemoglobinopathies are divided into two large groups: thalassemias and abnormal hemoglobins [8].

Violation of the biosynthesis of the globin structure leads to abnormal hemoglobins. To date, more than 400 abnormal hemoglobins are known that affect the structure of α -, β -, γ - and δ -polypeptide chains of globin. From a clinical point of view, stable abnormal hemoglobins are of greatest importance: hemoglobin S - the sickle cell gene, hemoglobin C, hemoglobin D, hemoglobin E and hemoglobin H. In addition to hemoglobin H, which is an abnormal variant of the globin α -polypeptide chain, other stable abnormal hemoglobins are an abnormal variant of the β -polypeptide chain of globin [6].

Materials and methods of research

In our study, we used the blood of 296 newborns for analysis, while the parents of children who were diagnosed with the disease also participated in the study. It should be noted that newborns were selected selectively, with the consent of their parents.

To determine the frequency of occurrence of hereditary hemoglobinopathies among newborns, we used the analytical method of isoelectric focusing (IEF) on polyacrylamide-ampholine plates with pH 3.5-9.5 and 5.5-8.5. This method allows you to divide the hemolysate prepared from the blood of newborns into the following nine hemoglobin fractions: HbA₁, HbA_{1c}, HbA₂, HbA₃, HbF₁, HbF₂, methemoglobin, intermediate methemoglobin (as two fractions). The most anodic is HbA₃, cathodic HbA₂.

From the anode to the cathode, hemoglobin fractions are arranged as follows; HbA₃, HbA_{1c}, HbA₁, HbF₁, HbF₂, methemoglobin intermediate, methemoglobin and HbA₂. Abnormal HbS (sickle cell carriage), HbD is located between the fractions of hemoglobins HbF₁ and intermediate methemoglobin. Abnormal HbE is located between the fractions of the intermediate methemoglobin and methemoglobin. Abnormal HbC is located at the level of the minor fraction of HbA₂.

To identify the type of mutations in thalassemia, genomic DNA was isolated from the venous blood of newborns, then the integrity of genomic DNA was analyzed by electrophoresis on agarose plates using certain synthetic oligonucleotide probes (primers). The type of mutation was determined by high-temperature allele-specific amplification.

Results of own research

Genetic screening of newborns for hereditary hemoglobinopathies made it possible to identify heterozygous carriage of thalassemia in 9 out of 296 examined newborns: α -thalassemia was detected in 5 newborns and β -thalassemia was detected in 4 newborns with a phenotypic frequency of 1.689% and 1.351%, respectively. Table 1 summarizes the results of this study.

| Number of newborns | α - thalassemia | | β - thalassemia | | HbA ₁ (%) | HbA ₂ (%) | HbA ₃ (%) | HbA ₄ (%) | HbA ₅ (%) | Met-Gemo Globin | Intermediate fraction of Methemoglobin (%) |
|--------------------|------------------------|-------|-----------------------|-------|----------------------|----------------------|----------------------|----------------------|----------------------|-----------------|--|
| | Quantity | % | Quantity | % | | | | | | | |
| 296 | 5 | 1,689 | 4 | 1,351 | 16,529,8 | 0,971,65 | 1,32,0 | 28,551,0 | 2,95,0 | 1,02,2 | 3,55,0 |

Table 1: Results of genetic blood screening of newborns for α - and β -thalassemia and levels of hemoglobin and methemoglobin fractions

On the plates of the IEF in newborns with heterozygous β -thalassemia, an increase in the level of HbA2 was observed compared with the results obtained with the IEF of the blood of normal - non-carriers of the pathological gene. In newborns who do not carry the pathological β -thalassemia gene, the HbA2 level is low and amounts to 0.03-0.05% of total hemoglobin, while in newborns with heterozygous β -thalassemia, the HbA2 level exceeded normal values and amounted to 0.97-1, 65%. This indicator is the only test we use to diagnose heterozygous β -thalassemia. Using the usual method of electrophoresis on cellulose acetate films, the heterozygous state of β -thalassemia is difficult due to the low sensitivity of the method.

In all 4 newborns, increased values of this minor fraction were observed. It should be noted that HbA2 values in adults with heterozygous β -thalassemia, in contrast to newborns, are higher and vary within 3.5-8.0%. Table 1 presents numerical values in percent levels of hemoglobin fractions, methemoglobin and intermediate fraction of methemoglobin.

As can be seen from Table 1, the levels of other hemoglobin fractions: HbA1, HbA3, HbF1, HbF2, methemoglobin and intermediate methemoglobin fraction were within normal limits.

In 5 newborns, the α -thalassemia gene, as in the case of β -thalassemia, had a heterozygous carriage. On the electropherogram closer to the anode in the blood of newborns with heterozygous carriage of α -thalassemia, in addition to normal hemoglobin fractions, two minor fractions were observed. These minor hemoglobin fractions, taking into account their isoelectric points in the range of pH-6.8-6.9, corresponded to hemoglobin fractions - Barts. The levels of hemoglobin fractions of Barts ranged from 2.8-5.4% and averaged 3.9%.

Unlike the β -globin gene, the α -globin gene is located on the 16th chromosome and is represented by four copies. Whereas the β -globin gene is represented by two copies. The α -globin genes are identical in structure and differ only in the number of synthesized α -polypeptide chains, i.e. differ in gene expression.

As a result of genetic screening of newborns for abnormal hemoglobins in 3 children of different sexes (2 boys, 1 girl), heterozygous carriage of structurally abnormal hemoglobins of the HbS and HbD types was revealed: 2 cases with sickle cell carriage (HbAS) and one case with HbD. This information is detailed in Table 2.

Table 2: Genetic screening results newborns for abnormal hemoglobins and results of methemoglobin and intermediate methemoglobin levels in the blood of newborns.

| Number of newborns | Anomal Hemoglobins | | | | | | | | Methemo-globin | | Intermediate Mehemo-globin | |
|--------------------|--------------------|------|----------|------|----------|---|----------|---|----------------|-------|----------------------------|-------|
| | HbS | | HbD | | HbE | | HbC | | | | | |
| | Quantity | % | Quantity | % | Quantity | % | Quantity | % | Quantity | % | Quantity | % |
| 296 | 2 | 0,75 | 1 | 0,37 | - | - | - | - | 13 | 4,391 | 11 | 3,716 |

Methemoglobinemia is caused by an increased content of methemoglobin, which is not able to carry oxygen. Allocate hereditary and acquired methemoglobinemia. Hereditary include congenital enzymopenic methemoglobinemia, caused by a sharp decrease or complete absence in erythrocytes of the activity of the enzyme lipoamide dehydrogenase, a dependent methemoglobin reductase, and hereditary, developing as a result of the presence of unstable or abnormal hemoglobins (M-hemoglobinopathies), in which, as a result of gene mutations, amino acid replacement has occurred residues in the globin polypeptide chains. Hereditary fermentopenic methemoglobinemia is inherited in an autosomal recessive manner, M-methemoglobinemia in an autosomal dominant manner. With methemoglobinopathy, immediately after the birth of a child, cyanosis is detected on the skin and mucous membranes, especially in the lips, nose, earlobes, and in the nail bed. It persists throughout life, increasing with cooling, taking methemoglobin-forming drugs [7, 12].

As a result of screening the levels of methemoglobin and intermediate methemoglobin in the blood of newborns, in 13 cases in heterosexual newborns, an increase in methemoglobin was observed, in 11 cases an increase in the intermediate fraction of methemoglobin. The quantitative and percentage results of this study are presented in table 2.

As can be seen from table 2, in 13 (4.391%) cases in heterosexual newborns, an increase in methemoglobin was observed, in 11 (3.716%) cases, an increase in the intermediate fraction of methemoglobin.

Results of molecular diagnostics of the β -thalassemia gene

To identify the type of mutations in β -thalassemia, genomic DNA was isolated from the venous blood of 4 newborns with a diagnosis of heterozygous β -thalassemia and their parents, then the integrity of genomic DNA was analyzed by electrophoresis on agarose plates. The type of mutation in β -thalassemia was determined by high-temperature allele-specific amplification. For this, the following synthetic oligonucleotide probes (primers) were used: β_0 -IVS-2-1 (G-A), β_0 -kodon 8 (-AA); β_+ -IVS-1-110 (G-A); β_0 -kodon 8/9 (+G); β_+ -IVS-1-6 (T-C) and β_+ -IVS-1-5 (G-C).

Each DNA sample isolated from the blood of newborns was hybridized with primers separately. Of the six primer samples, a positive signal was obtained for two and two types of mutations for the β -thalassemia gene were identified:

1. replacement of the guanine nucleotide with the adenine nucleotide in position 1 of the second large intron of the β -globin gene with the β_0 -thalassemia phenotype - β_0 -IVS-2-1 (G-A);

2. replacement of the nucleotide guanine with the nucleotide adenine in position 110 of the first small intron of the β -globin gene with the β_+ -thalassemia phenotype, β_+ -IVS-1-110 (D-A).

Figure 1 shows an electrophoregram of PCR diagnostics with a mutation replacing the nucleotide guanine with the nucleotide adenine in the 1st position of the second - large intron of the β -globin gene with the β_0 -thalassemia phenotype - β_0 -IVS-2-1 (G-A).

As can be seen from figure 1, out of ten DNA samples, in one case (No.4) a mutation was identified - the replacement of the nucleotide guanine with the nucleotide adenine in the 1st position of the second - a large intron of the β -globin gene with the phenotype of β_0 -thalassemia. A positive signal was obtained for one sample under No.4. A negative signal was obtained for samples Nos. 1,2,3,5,6,7,8,9 and 10.

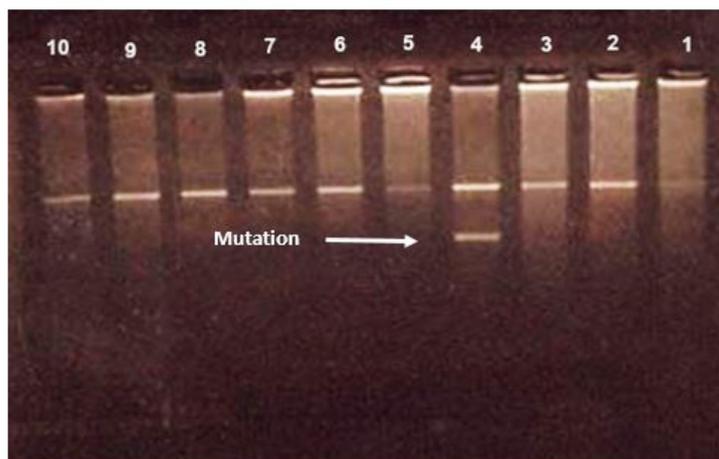


Figure 1: Electrophoregram of PCR diagnostics with mutation - β_0 -IVS-2-1 (G-A).

As can be seen from Figure 2, out of ten DNA samples, in two cases (No.2 and No.6), a mutation was identified - the replacement of the nucleotide guanine with the nucleotide adenine in the 110th position of the first small intron of the β -globin gene with the phenotype of β_+ -thalassemia. A positive signal was obtained for two samples: number 2 and number 6. A negative signal was

Figure 2 shows an electrophoregram of PCR diagnostics with a mutation from the guanine nucleotide to the adenine nucleotide in position 110 of the first small intron of the β -globin gene with the β^+ -thalassemia phenotype - β^+ -IVS-1-110 (D-A).

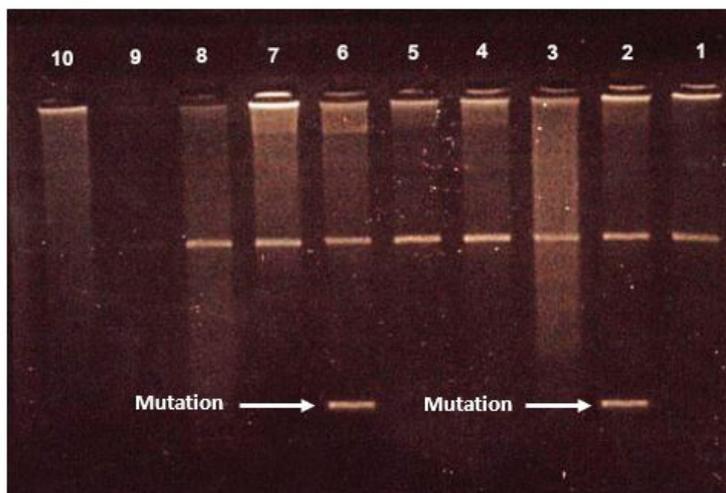


Figure 2: Electrophoregram of PCR diagnostics with mutation - β^+ -IVS-1-110 (G-A).

obtained for samples numbered 1,3,4,5,7,8,9 and 10. At the same time, two types of β -thalassemia mutations were identified: the replacement of the guanine nucleotide by adenine nucleotide in the intron part of the β -globin gene - β^+ -IVS-1-110(G-A) and β^0 -IVS-2-1(G-A). It was not possible to reveal a heterozygous carriage of β -thalassemia in the parents of three examined patients.

Findings

According to the genetic screening for hereditary hemoglobinopathies among newborns in Baku, the presence of pathological genes for α - and β -thalassemia and structurally abnormal hemoglobins S and D was found, which is directly related to further disorders of the hematopoietic system leading to anemia in children. The obtained results of genetic screening will allow pediatricians to timely treat sick children before the manifestation of the disease clinic. The introduction of programs of mass screening of newborns plays an important role in the early detection of hereditary diseases and the timely appointment of appropriate therapy. The identification and treatment of children through mass screening programs also helps prevent the development of severe mental and other disabilities.

Conclusion

The current level of development of genetics and medicine in general allows for timely medical and genetic counseling of families with the presence of pathological genes in spouses (a family with a genetic risk) in order to prevent a homozygous state in a child.

Screening not only allows you to identify the risk of manifestation of chromosomal abnormalities in a child, but also to diagnose and prescribe timely therapy for various complications of pregnancy. Prevention of complications identified through screening helps prevent many complications of the last trimester of pregnancy, and possibly save the life and health of the baby. It is also recommended to conduct invasive and non-invasive prenatal diagnosis in the fetus in the first and second trimesters of pregnancy.

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